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Short communication

Diagnostic interaction between bovine tuberculosis (bTB) and Johne's disease in bTB highly prevalent dairy farms of Uruguay

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ABSTRACT

The consolidation of the dairy industry, with increase in sizes, density and productivity of the herds, was associated to unprecedented bovine tuberculosis (bTB) prevalence levels in dairy herds in Uruguay, where Johne's disease (JD), another mycobacterial disease, is also prevalent. Here, we aimed to characterize the association between bTB- and JD-diagnostic results in two heavily bTB- and JD-coinfected dairy herds. Results from bTB-intradermal tests and JD-ELISA in 686 cows indicated a significantly ($P < 0.001$) higher frequency of bTB-positive animals in the JD-positive population, in which a significantly lower agreement between the caudal and cervical comparative intradermal tests was observed, compared to the JD-negative population. These findings suggest a significant association between the detection of these mycobacterial diseases, that may affect the performance of the routine bTB diagnostic tests performed in dairy herds in Uruguay.

Bovine tuberculosis (bTB) is a worldwide-distributed chronic infectious disease of cattle caused mainly by infection with *Mycobacterium bovis (M. bovis)*, which represents a threat to animal and public health. The annual number of bTB-positive detected herds, the within-herd prevalence in infected herds, and the time from detection-to-control[1] has increased over the last decade in Uruguay, despite measures implemented as part of the national bTB-control program. Briefly, dairy animals are annually tested with the caudal fold tuberculin test (CFT) and reactors are confirmed with the comparative cervical tuberculin test (CCT). CCT reactors are sent to slaughter and the complete herd is re-tested every 60 to 120 days until herd have two consecutive negative herd-tests are achieved. Challenges in controlling the disease may be explained, at least in part, by the consolidation of the dairy industry[2], given that large dairy herds (>360 animals) with frequent movement of animals (>44 individuals annually) have become relatively common in the country. Those unusually large dairy farms have been found to experience a high risk of bTB-breakdown, what could be related to the limited efficacy of control strategies in place, that were initially designed for a more traditional dairy production standards characterized by relatively small farms, with less animal density, less individual production pressure, and infrequent movements (Picasso et al., 2017).

Insufficient accuracy of the bTB-diagnostic tests routinely used in Uruguay (caudal fold test –CFT- followed by the comparative cervical test –CCT- for confirmation of reactors), represents an additional constraint to the success of the bTB control program. This lack in performance may be explained in part by the cross-reactivity with other mycobacterial infections, such as *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the etiological agent for Johne's disease (JD), particularly in the case of the CFT (Brito et al., 2014). In Uruguay, JD is widespread in dairy cattle, with a reported within-herd prevalence of 2.5% in 2015 (Suanes et al., 2018).

In the current changing scenario of the Uruguayan dairy industry, with untraditional large herds, frequent animal movements, and widespread occurrence of JD, there is a need to assess the performance of the bTB-diagnostic strategies (CFT, and CCT), and their potential interaction with JD-diagnosis, with the ultimate objective of evaluating and informing the design of the control plan for the disease. Here, we aimed to characterize and estimate the association between bTB- and

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[1] See: http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation

[2] See https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2018/Anuario_2018.pdf

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Table 1
Bovine tuberculosis and Johne's disease (JD) diagnostic results in dairy cows selected.

JD-ELISA	Number of samples (%)	Number of cows positive to CFT[a] (%)	Number of cows positive to CCT[b] (%)	Cohen's kappa[c] (95%CI)
Positive	303 (44.16)	260 (85.81)	167 (55.11)	0.34 (0.25–0.42)
Negative	383 (55.84)	167 (43.60)	81 (21.81)	0.52 (0.44–0.59)
Total	686	427 (62.24)	248 (36.15)	0.46 (0.51–0.56)

[a] Caudal Fold Tuberculin test (CFT).
[b] Comparative Cervical Tuberculin test (CCT).
[c] Agreement between CFT and CCT.

JD-diagnostic results in two farms with the recently identified bTB risk factors in which both diseases were present at high prevalence.

We randomly sampled 686 Holstein cows (>24 months) from two large (~1000 animals, >75th percentile for the country[2]) dairy herds from one of the largest dairy companies in Uruguay. These two herds had been bTB-infected since 2013, had a history of JD-seropositive results and animals with chronic diarrhoea, were located in the same geographic region, mingled their animals frequently and systematically, had similar management practices, and were subjected to similar bTB-control measures, which are mandatory for bTB-infected herds in Uruguay[3].

Selected animals were subject to bTB-intradermal testing which involves the inoculation of purified protein derivate from *M. bovis* (PPDb) for CFT, and from *M. bovis* and *M. avium* (PPDa) for the CCT. The CFT was performed in all animals, and those with a palpable increase in skin thickness or with local clinical signs of inflammation 72 h post inoculation were re-tested using the CCT within the following 10 days. Animals with >4 mm increase difference in skin thickness between the PPDb- and PPDa- inoculation site after 72 h were considered bTB-infected2 . Additionally, serum samples were collected from the coccygeal vein of all animals when the CFT was interpreted to perform a JD-indirect Enzyme-Linked Immunosorbent Assay (ELISA) (ID.Vet, Montpellier, France) following the manufacturer recommendations; animals with a sample-to-positive ratio result (S/P) ≤ 0.6 were considered negatives and otherwise were classified as positives.

Most animals (427/686, 62.24%) were positive to the CFT, and of that 58.08% (248/427) were also positive to the CCT. In addition, 44.16% (303/686) animals tested positive to the JD-ELISA, confirming the high bTB- and JD- prevalence. Interestingly, the proportion of bTB-positive animals (to both CFT and CCT) was significantly ($P < 0.001$) higher among JD-reactors compared to JD-negative cattle (Table 1). The degree of diagnostic interference in bTB- and JD-diagnostic tests in animals infected with either or both pathogens under field conditions is still not fully understood (Aranaz et al., 2006; Brito et al., 2014; Dunn et al., 2005). Results here may indicate that animals infected with one of the diseases would develop a cross-reactive immune response to the other disease, increasing the sensitivity of the other test or reducing its specificity (Alvarez et al., 2009; Lilenbaum et al., 2007; Roupie et al., 2018), what may have happened here, with a decreased specificity of the JD-ELISA (Dunn et al., 2005). Other potential explanation may be an increased individual susceptibility (or resistance) to mycobacterial infections in which animals that are infected with one disease are/become more susceptible to the other.

The agreement between the two bTB intradermal tests (CFT and CCT) was fair in the JD-ELISA-positive animals (kappa-coefficient = 0.34, CI: 0.25–0.42) and moderate in the JD-ELISA-negative population (kappa-coefficient = 0.52, CI:0.44–0.59), with a lower agreement in the JD-positive animals than the overall agreement observed (Table 1). The main source of disagreement between the results in the two intradermal tests in the JD-positive group was the higher proportion of CFT-positive/CCT-negative results (0.31 compared with 0.22 among the JD-negative animals). Such difference suggests that JD-seropositivity has an impact on the result of the skin tests, by either reducing CFT-specificity or CCT-sensitivity. Potential explanations for a higher rate of CFT-false positive results include the cross-reactivity between bTB and JD, with the subsequent CFT-specificity reduction that may only be partially resolved with the use of the CCT. A reduction in the CCT-sensitivity, on the other hand, could be explained by the induction of a greater response to the PPDa inoculation that would mask the PPDb reaction, leading to CCT-false negative results in bTB-infected animals (Hope et al., 2005). The potential impact of this phenomenon in smaller infected herds in which the number of reactors would be lower should be assessed. Still, in a scenario of high prevalence of bTB as the one evaluated here the reduction in the CCT-sensitivity (and therefore of its negative predictive value) is the most concerning possibility, and highlights the necessity to re-evaluate the use of testing in series to control bTB in high bTB-prevalent and JD-coinfected herds in Uruguay.

This study is the first to characterize the diagnostic interaction between bTB and JD, the two most important mycobacterial diseases in Uruguayan cattle, in high prevalence, coinfected herds. Most importantly, our findings suggest that evaluating the distribution of both diseases in high prevalence bTB-infected herds in Uruguay may be important to facilitate disease control and eventual eradication.

Conflict of interest statement

Authors declare that this research was conducted in the absence of any financial, commercial or personal relationship that could result in a conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2019.100052.

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